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Review

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Epigenetic Modifications in Schizophrenia and Related Disorders: Molecular Scars of Environmental Exposures and Source of Phenotypic Variability

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ABSTRACT

Epigenetic modifications are increasingly recognized to play a role in the etiology and pathophysiology of schizophrenia and other psychiatric disorders with developmental origins. Here, we summarize clinical and preclinical findings of epigenetic alterations in schizophrenia and relevant disease models and discuss their putative origin. Recent findings suggest that certain schizophrenia risk loci can influence stochastic variation in gene expression through epigenetic processes, highlighting the intricate interaction between genetic and epigenetic control of neurodevelopmental trajectories. In addition, a substantial portion of epigenetic alterations in schizophrenia and related disorders may be acquired through environmental factors and may be manifested as molecular "scars." Some of these scars can influence brain functions throughout the entire lifespan and may even be transmitted across generations via epigenetic germline inheritance. Epigenetic modifications, whether caused by genetic or environmental factors, are plausible molecular sources of phenotypic heterogeneity and offer a target for therapeutic interventions. The further elucidation of epigenetic modifications thus may increase our knowledge regarding schizophrenia's heterogeneous etiology and pathophysiology and, in the long term, may advance personalized treatments through the use of biomarker-guided epigenetic interventions.

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Schizophrenia is a chronic and severe mental disorder that affects approximately 1 in 100 individuals worldwide (1). It is characterized by varying degrees of cognitive impairments, emotional aberrations, and behavioral anomalies, which together undermine basic processes of perception, reasoning, and judgment. Typically, the onset of full-blown schizophrenia is in early adulthood and includes a myriad of symptoms, which are referred to as positive symptoms (e.g., visual and/or auditory hallucinations, delusions, paranoia, psychomotor agitation), negative symptoms (e.g., social withdrawal, apathy, deficits in motivation and reward-related functions), and cognitive symptoms (e.g., deficits in executive functioning, working memory, and attention) (2).

The etiology of schizophrenia is likely to be multifactorial, with multiple small-effect and fewer large-effect susceptibility genes interacting with several environmental factors (2–4). The combination of genetic and environmental risk factors is believed to affect the normal course of brain development and maturation, manifesting in a cascade of neurotransmitter and circuit dysfunctions and impaired connectivity in early adulthood (5,6). While common and rare genetic abnormalities provide the basis for the substantial heritability of schizophrenia, environmental factors may contribute to the disorder's etiology partly through epigenetic processes (7,8). The latter are commonly referred to as the combination of mechanisms that confer short- and long-term changes in gene expression without altering the DNA code itself (7,8). Epigenetic processes include several interrelated mechanisms such as chromatin remodeling, histone modifications, DNA methylation (DNAm), and expression of noncoding RNAs (ncRNAs) (Figure 1 and Supplement). The orchestrated action of these epigenetic processes has a considerable influence on gene expression and may further provide a basis for transgenerational nongenetic inheritance of environmentally acquired traits (9).

While epigenetic processes have long been speculated to contribute to monozygotic twin discordance (10), their importance is increasingly recognized for virtually every aspect of normal and pathological brain development and functioning (11). Not only may epigenetic processes mediate the effects of environmental risk in schizophrenia and other multifactorial neuropsychiatric disorders, but also they can interact with the genomic risk associated with these disorders (12,13). Hence, the exploration of epigenetic modifications holds the promise of new discoveries at the crossroads of genes and environment. In this review article, we summarize clinical and

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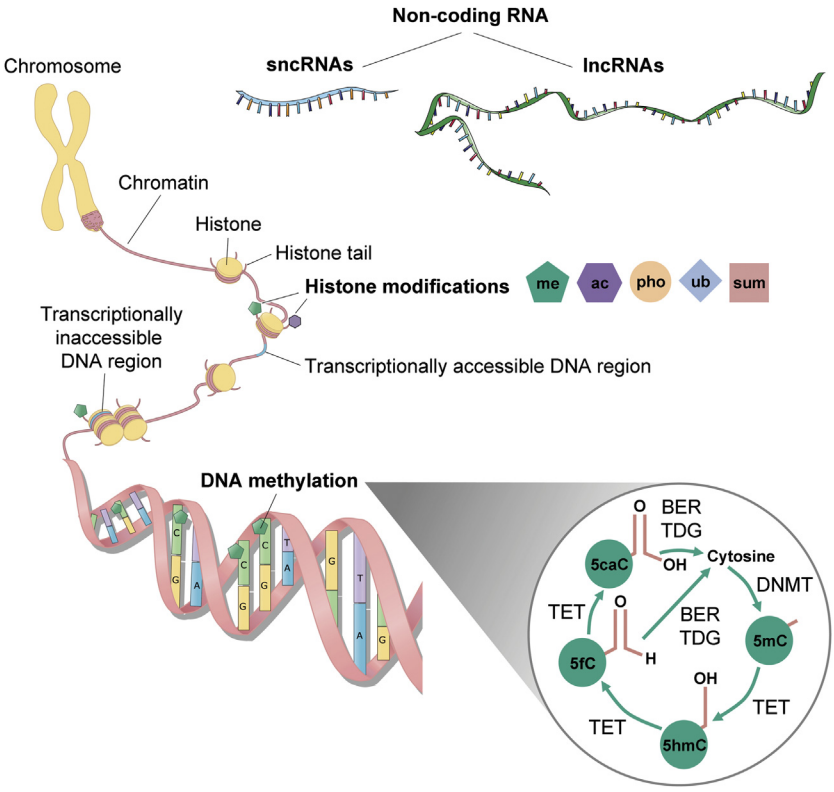

Figure 1. Schematic representation of chromatin structure and major epigenetic processes. The DNA–protein complex within chromosomes is referred to as chromatin. The functional unit of the chromatin is the nucleosome (not shown), which is composed of DNA wrapped around a core octamer of histone proteins. The DNA–histone interaction occurs at the N-terminal tails of these histones, which provide sites for epigenetic marks known as histone modifications. A number of histone modifications exist, including methylation (me), acetylation (ac), phosphorylation (pho), ubiquitination (ub), and SUMOylation (sum). Histone modifications determine the extent to which chromatin is wrapped around histone proteins. Loosely coiled chromatin contains transcriptionally accessible DNA regions, whereas tightly coiled chromatin comprises transcriptionally inactive DNA regions. DNA methylation refers to the covalent modification of the DNA at position 5′ in the cytosine ring (5mC), which is found primarily at CpG dinucleotides. 5mC (and other methylation-related epigenetic marks) can also exist at non-CpG sites (not shown). Whereas 5mC is established and maintained by methyltransferases (DNMTs), it is oxidized by the TET family of dioxygenase proteins to 5-hydroxymethylcytosine (5hmC). In successive steps, TET enzymes further hydroxylate 5hmC to generate 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Whereas 5fC and 5caC are recognized and removed by TDG, the created abasic site is repaired by the BER pathway, generating an unmodified cytosine. Besides epigenetic modifications at histone proteins and DNA, noncoding RNAs provide an additional level of epigenetic regulation involved in chromatin and nuclear remodeling, gene transcription, translational repression, and degradation of messenger RNAs. Based on their size, noncoding RNAs can be broadly subdivided into lncRNAs (>200 nucleotides) and sncRNAs (<200 nucleotides), the latter of which contain small inhibiting RNAs, microRNAs, PIWI-interacting RNAs, and small nuclear RNAs (not shown). BER, base excision repair; DNMT, DNA methyltransferase; lncRNAs, long noncoding RNAs; sncRNAs, short noncoding RNAs; TDG, thymine-DNA glycosylase; TET, ten-eleven translocation.

preclinical findings of epigenetic alterations in schizophrenia and relevant disease models and discuss their putative origin. We also speculate how such modifications may contribute to pathological trait variability and offer avenues for novel treatments.

EPIGENETIC MODIFICATIONS IN SCHIZOPHRENIA

DNA Methylation

Alterations in DNAm were among the first epigenetic modifications to be associated with schizophrenia. Pioneering work conducted by Costa, Guidotti, Grayson, and colleagues indicated that reduced transcription of the extracellular matrix protein reelin (*RELN*) in the brains of people with schizophrenia might be caused by hypermethylation of its promoter region (14–16). This notion was further supported by findings showing that patients with schizophrenia display increased cortical and subcortical expression of DNMT1 (17,18), which primarily functions to maintain DNAm at CpG sites (19). In addition to *RELN,* numerous other (historical) candidate risk genes for schizophrenia were found to be differentially methylated, including genes involved in gamma-aminobutyric acidergic (GABAergic) (*GAD1*) functions (20), dopaminergic (*DRD2, DRD3, COMT, IGF2*) functions (10,21–27), serotonergic (*HTR2A*) functions (28,29), and oligodendrocyte (*SOX10*) functions (30,31). However, some of these initial findings could not be replicated (32,33), which is likely explained by the use of small sample sizes and/or differences in the methods used to quantify DNAm in postmortem samples, with initial studies lacking technological sensitivity as compared with contemporary methods.

As a counterpart to targeted approaches, technical and theoretical advances have recently facilitated DNAm profiling across the entire genome (Table 1). These studies are based on sample sizes that range among 10 (34–37), 20 to 40 (32,38–44), and more than 60 (45–47) patients and control subjects. They are mostly based on the Infinium Human Methylation 450K BeadChip, which allows the user to assess more than 450,000 CpGs located throughout the genome, and they mainly focus on the prefrontal cortex (PFC) (32,34,37–39,41,42,44–47), the hippocampus (35–37,42), and, to a lesser extent, the striatum (42,43), cerebellum (38,40,42), and anterior cingulate cortex (37). The largest study to date, performed by Jaffe *et al.* (47), identified 2104 differentially methylated CpGs in the PFC of subjects with schizophrenia relative to control subjects. The differentially methylated CpGs were found to be enriched in genes relevant for embryonic development, cell fate commitment, and nervous system differentiation. The authors also observed that the epigenetic landscape, as represented by DNAm, varied markedly across

Table 1. Genome-wide DNA Methylation Changes in Schizophrenia: Brain Tissue

Sample	Brain Region	Method	Most Significant	Reference
35 SZ and 35 CTR	FC	CpG island microarray	Male subjects: *EXOSC7, GRIA2, ELMOD1, KCNJ6, WDR18* Female subjects: *C6orf84, HCG9, SLC17A7, NR4A2, AB051500*	(32)
20 SZ and 23 CTR	PFC (London)	450K array	*GSDMD, RASA3, HTR5A, PPFIA1, CACNA1G* (from DMPs associated with SZ)	(38)
21 SZ and 23 CTR	CBL (London)		*ANO4, NRN1, PPAPDC1B, NAIF1, SNX26* (from DMRs associated with SZ)	
18 SZ and 15 CTR	PFC (Montreal)		*NAV1, ZNF200, PRH2, NFIA, COL16A1* (from DMPs associated with SZ)	
24 SZ and 24 CTR	PFC	450K array	*TNR6C6, C21orf33, HOXA11, CCNI, HOXA13, NOS1, AKT1, DNMT1, SOX10, DTNBP1, PPP3CC*	(39)
106 SZ and 110 CTR	DLPFC	27K array	*ASTN2, IL1RL1, GRIA4, LRRIQ2, SH2BP1, ABCB1, CEACAM19*	(45)
39 SZ and 43 CTR	CBL	27K array	*PIK3R1, BTN3A3, NHLH1, SLC16A7*	(40)
62 SZ and 62 CTR	PFC	450K array	*CERS3, DPPA5, PRDM9, DDX43, REC8, LY6G5C* (DMRs)	(46)
5 SZ and 6 CTR	PFC	MeDIP-seq RNA-seq	*PLP1, NR4A1, IL1B, GFAP, APC, TAAR1, MYT1L, GRIP1, ASTN2, EGFR, CD28, SLC6A2*	(34)
8 SZ and 8 CTR	H (CA1 vs. CA2/3)	450K array	*MSX1, DAXX, CCND2, FOXG1, GAD1*	(35)
19 SZ and 3 CTR	H	450K array	*NUBP1, STK32B, AIG1, PRKCE, RASA3*	(37)
	DLPFC		*HLA-DQA1, HCN2, AJAP1, HLA-B, HLA-DRB5*	
	ACC		*C4orf50, GALNT1, VSX2, SAPS2, KCNK7*	
108 SZ and 136 CTR	DLPFC	450K array	*CD164, COPZ2, SUGT1, HAT1, TYW1B*	(47)
26 SZ and 27 CTR	PFC	MBD-seq	*FOXP1, IL1RAP, AKAP13, SLC39A11, RPTOR*	(41)
38 SZ and 38 CTR	PFC	450K array	*NCAM1, SND1, LILRB1, GSDMD, ABTB*	(42)
37 SZ and 45 CTR	STR		*SYNPO, HECW1, GRINL1A, DEFB115, GBP4*	
14 SZ and 13 CTR	H		*ATP6V0D, CACNA1G, RBM24, ZCCHC10, RASIP1*	
37 SZ and 40 CTR	CBL		*GART, ELK3, PDX1, ZNF586, ZNF217*	
8 SZ and 8 CTR	H (GABAergic interneurons)	450K array	*SLC7A6*	(36)
22 SZ and 22 CTR	STG	450K array	*DLG1 (or BDH1), SERGET, TPGS2, CLEC16A, AHRR; BAIAP2,* and *DLG1* strongly associated with dendritic spine density	(43)
23 SZ and 27 CTR	Brodmann area 46	WGBS	Cell type–specific whole-genome methylomes from neurons and oligodendrocytes obtained from SZ and CTR; DNA methylation differences tended to occur in cell type differentially methylated sites. 97 DM CpGs (14 NeuN+ and 83 OLIG2+ specific). Top NeuN+: *FSTL5, GNA14, ERC2, LHFPL2, OSBPL6*. Top OLIG2+: *PPP1R9A, CHN2, CBLN4, TRIM49B, MGC32805*	(44)

The table summarizes the main findings from studies assessing genome-wide DNA methylation profiles in brain tissue of patients with schizophrenia (SZ) relative to matched control subjects (CTR) and/or in subjects at high risk for psychosis relative to control subjects. The table specifies the study sample, brain region, method used to assess DNA methylation profiles, and genes and loci with the most significant DNA methylation changes.

ACC, anterior cingulate cortex; CA, cornu ammonis subfield of hippocampus; CBL, cerebellum; DMP, differentially methylated position; DMR, differentially methylated region; DLPFC, dorsolateral prefrontal cortex; FC, frontal cortex; GABAergic, gamma-aminobutyric acidergic; H, hippocampus; MBD-seq, methyl-CpG binding domain protein-enriched genome sequencing; MeDIP-seq, methylated DNA immunoprecipitation sequencing; PFC, prefrontal cortex; RNA-seq, RNA sequencing; STG, superior temporal gyrus; STR, striatum; WGBS, whole genome bisulfate sequencing.

development, possibly mirroring shifts in neuronal composition across the lifespan and age-dependent changes in gene expression profiles. Interestingly, schizophrenia risk loci were primarily enriched for loci expressing these shifting epigenetic states, particularly those that change during the transition from prenatal to postnatal life. Together, these data implicate an epigenetic component to the developmental origins of schizophrenia and suggest that there is an early epigenetic intermediate between genetically and biologically determined risks for the disorder (47).

There is also an increasing number of genome-wide DNAm profiling studies of non–central nervous system (CNS) tissues such as blood (12,13,48–53) and saliva (54) (Table 2). These can be further subdivided into longitudinal studies (48–51,54), which assessed markers of conversion to psychosis in twins discordant for schizophrenia or high-risk individuals, and biomarker studies (12,13,52,53), which aim at uncovering biomarkers for schizophrenia in large cohorts. One advantage of such studies is that they provide a means to perform noninvasive and longitudinal epigenetic examinations, which

Table 2. Genome-wide DNA Methylation Changes in Schizophrenia: Peripheral Tissue

Sample	Tissue Type	Method	Most Significant	Reference
Longitudinal Studies				
11 MZ twin pairs discordant for SZ	Whole blood	27K array	*PUS3, SYNGR2, KDELR1, PDK3, PPARGC1A*	(48)
2 MZ twin pairs discordant for SZ	Whole blood	NimbleGen 720K array	*HIST2H2AA3, HIST2H2AA4, HIST2H3A, HIST2H3Ac AK2* (pair 1) *DVL1, KIAA1751, CEP104, DFFB, CAMTA1* (pair 2)	(49)
24 MZ twin pairs discordant for psychotic symptoms at 12 years of age	Buccal cells	450K array	*C5orf42, MORF4L1, GALNTL4, LOC340094, DIP2C*	(54)
14 converters and 25 nonconverters to psychosis	Whole blood	450K array	*NRPI1, CHL1, EPHNA3, IL17RE, AKT1*	(50)
767 subjects with psychotic episodes at 12 and 18 years of age (ALSPAC cohort)	Cord blood and whole blood at 7 and 15 to 17 years of age	450K array	*BAIAP2, F2RL1, FAM19A5, FGFR3, LFNG, LPR5, MAD1L1, OLFML2A, RNH1* (detected in more than one comparison)	(51)
Biomarker Studies				
759 SZ and 738 CTR discovery cohort; 178 SZ and 182 CTR replication cohort 1; 561 SZ and 582 CTR replication cohort 2	Whole blood	MBD-seq	*FAM63B, ARHGAP26, CTAGE11P, TBC1D22A, RELN* (replicated in all cohorts)	(52)
353 SZ and 322 CTR discovery cohort; 414 SZ and 433 CTR replication cohort	Whole blood	450K array	*FAM126A, PPTC7, GYG1, SIK3, USP36* (part of 25 DMPs replicated also in the second cohort)	(12)
689 SZ and 645 CTR discovery cohort; 247 SZ and 250 CTR replication cohort	Whole blood	450K array	*RPS6KA1, MGRN1, S100A2, NCOR, KIAA0355* (part of the top 15 replicated in all cohorts)	(13)
130 adolescents at risk for psychiatric disorders, discovery cohort; 93 adolescents, replication cohort	Whole blood	450K array	305,147 CpG loci investigated in relation to 37 SNPs associated with psychiatric disorders. 5 SNPs (in *HCRTR1, GAD1, HDAC3*, and *FKBP5*) associated with 7 DMPs (closest gene *PEF1*, *GAD1*, *DIAPH1*, *PCDHGC3*, *TULP1*, and *C11orf9*)	(53)

The table summarizes the main findings from studies assessing genome-wide DNA methylation profiles in peripheral tissues of patients with schizophrenia (SZ) relative to matched control subjects (CTR) and/or in subjects at high risk for psychosis relative to control subjects. The table specifies the study sample, type of peripheral tissue, method used to assess DNA methylation profiles, and genes and loci with the most significant DNA methylation changes.

ALSPAC, Avon Longitudinal Study of Parents and Children; DMP, differentially methylated position; MBD-seq, methyl-CpG binding domain protein-enriched genome sequencing; MZ, monozygotic; SNP, single nucleotide polymorphism.

would not be possible using brain tissue. Peripheral epigenetic profiling, however, does not necessarily capture disease-associated epigenetic changes occurring in the CNS. In support of the latter notion, there is only minimal overlap between the findings obtained by genome-wide DNAm profiling in CNS and non-CNS tissues of schizophrenia (Tables 1 and 2). Hence, while peripheral epigenetic profiling may still be valid for the identification of molecular biomarkers, it appears that peripheral epigenetic signatures do not grossly mirror those in the CNS and vice versa.

The assay for transposase-accessible chromatin using sequencing (ATAC-seq) is a powerful addition to the genome-wide DNAm profiling approaches because it allows researchers to assess genome-wide chromatin accessibility and overall changes in epigenetic landscapes and corresponding gene signatures (55). ATAC-seq has recently been implemented in schizophrenia research (56,57) and has already provided preliminary evidence for altered chromatin accessibility in schizophrenia cases compared with control cases. For example, performing ATAC-seq on adult PFC brain samples from 135 individuals with schizophrenia and 137 control subjects, Bryois *et al.* (56) found that chromatin accessibility in schizophrenia differs in three specific genomic regions. Moreover, the authors identified 118,152 ATAC-seq peaks in PFC tissue, many of which were found to be enriched for schizophrenia single nucleotide polymorphism heritability (56). Further use of ATAC-seq is expected to provide imperative new knowledge regarding the molecular mechanisms of altered gene expression in schizophrenia and beyond.

Histone Modifications

Several lines of evidence suggest that histone post-translational modifications (PTMs) play a role in the etiology and pathophysiology of schizophrenia. Initial support for this notion stemmed from the observation that valproate, a mood stabilizer with frequent off-label use in schizophrenia (58), is a histone deacetylase (HDAC) inhibitor when administered at therapeutic doses (59). Based on the findings showing beneficial effects of valproate on clinical symptoms in some patients with schizophrenia (60), various studies explored whether schizophrenia is linked to specific histone PTMs and associated alterations in the enzymes that catalyze such modifications. The existing evidence for such alterations includes the presence of high levels of *H3R17* methylation (61), increased cortical levels of HDAC1 (62,63), and histone methyltransferases (64,65), but reduced cortical levels of HDAC2 (66) in postmortem samples of cases with schizophrenia. The latter finding was recently corroborated by an in vivo positron emission tomography study using a radiotracer version of the potent HDAC inhibitor [^{11}C]Martinostat, showing

lower in vivo HDAC levels in cases with schizophrenia as compared with matched control subjects (67). Furthermore, this study revealed a positive correlation between cortical HDAC levels and cognitive performance independent of diagnostic groups (67), highlighting a general involvement of HDACs in cognitive functions.

Imperative new research efforts, mainly fueled by the PsychENCODE initiative (68), are also being made to comprehensively map histone PTMs across the genome in different brain regions, cell types, and psychiatric disorders. This initiative aims at providing a public resource of genomic data (including histone PTMs) originating from tissue and cell-specific samples of 1866 individuals (558 of whom were diagnosed with schizophrenia). Based on the practical guidelines for chromatin immunoprecipitation sequencing followed by deep sequencing suggested by Kundakovic *et al.* (69), which ensure a high degree of standardization across studies, two studies investigated genome-wide histone PTMs in health and disease. In a first study, Girdhar *et al.* (70) profiled open chromatin-associated modifications in neuronal and nonneuronal cells isolated from the dorsolateral PFC and the anterior cingulate cortex of healthy subjects. These investigations aimed at generating reference maps for two histone marks (H3K4me3 and H3K27ac) that are associated with active promoters and enhancers, which in turn can be used as a resource to map histone PTMs in a cell-type-specific manner. The results obtained by Girdhar *et al.* (70) show that neuronal and nonneuronal cell types strongly differ with regard to histone modification profiles, highlighting the critical role of cell-type-specific epigenetic signatures in cortical tissue. Moreover, the study found that risk variants for schizophrenia were overrepresented in neuronal versus nonneuronal H3K4me3 and H3K27ac landscapes (70), emphasizing the need to focus on neuronal populations for future investigations of histone PTMs in this disorder.

In a second study, Wang *et al.* (71) annotated active enhancers (defined as open chromatin regions depleted in H3K4me3 and enriched in H3K27ac) in neuronal and nonneuronal nuclei originating from healthy individuals and patients with schizophrenic. This impressive dataset was used to calculate chromatin quantitative trait loci and integrated with other genomic data to obtain regulatory networks linking enhancers, transcription factors, and target genes. These regulatory networks were then further used to link schizophrenia genome-wide association study variants to genes (uncovering a set of 321 novel high-confidence schizophrenia-associated genes) and finally embedded into a deep learning model to predict psychiatric phenotypes from genomic data, which provides a 6-fold increased improvement in prediction over additive polygenic risk scores (PRSs) (71).

A few studies further identified altered histone PTMs in peripheral tissues from patients with schizophrenia. For example, reduced acetylated H3 levels (72) and higher levels of H3K9me2 (73) were found in lymphocytes obtained from patients relative to control subjects. Intriguingly, increased H3K9me2 levels were also found in the parietal cortex of schizophrenia cases (64,65), suggesting that there is a certain correspondence between altered histone PTMs in peripheral and central tissues.

Noncoding RNAs

The majority of studies examining the possible involvement of ncRNAs in schizophrenia have focused on microRNAs (miRNAs) as opposed to other ncRNAs (34,74,75). A likely reason for the strong focus on miRNAs is that a microdeletion at chromosome 22q11.2, which is a genetic variation conferring high risk of schizophrenia and other neurodevelopmental disorders, includes a gene (*DGCR8*) encoding for an miRNA processing protein (76). This discovery led to the hypothesis that miRNA dysfunctionalities, whether induced by genetic or environmental factors, are involved in the etiology and pathophysiology of schizophrenia (77). Since the seminal findings of Perkins *et al.* (78), who were the first to identify altered miRNA profiles in postmortem PFC from patients with schizophrenia, a number of studies revealed altered cortical and subcortical expression of various miRNAs, including mir130 (79), mir181b (80), mir497 (81), mir185 (82), mir9 (83), mir195, mir301a (84), mir132, mir1307 (75), and mir137 (85). Notably, mir137 was found to be linked to schizophrenia in an early genome-wide association study (86), a finding that was confirmed in the largest genome-wide association study of schizophrenia existing to date (4). In further support of its involvement in schizophrenia, mir137 represents an important signaling node in various gene networks relevant to brain development and functions, including axon guidance signaling, ephrin receptor signaling, and synaptic activity (87,88), all of which are known to be disrupted in schizophrenia and related disorders (2–5).

miRNAs have also been profiled in the blood of patients with schizophrenia (89), with the main aim of identifying blood biomarkers of the disease. In a recent genome-wide expression study, Du *et al.* (90) found that several miRNAs were differentially expressed in serum exosomes of patients with first-episode, drug-free schizophrenia relative to matched control subjects. Importantly, among the differentially expressed miRNAs, 11 could be used to dissociate schizophrenia samples from control samples with 75% to 90% accuracy (90). Despite this remarkable precision, however, blood miRNA profiling tends to produce equivocal results in the context of schizophrenia, with biomarker candidates varying across independent studies (91–93). While this variability may arise from various cofounding effects, including differences in the type of tissue, low sample size, and smoking-related epigenetic effects (94), it may also mirror the disorder's etiological (and phenotypic) heterogeneity (95,96).

EPIGENETIC MODIFICATIONS AS MOLECULAR SCARS OF ENVIRONMENTAL EXPOSURES

While certain epigenetic modifications in schizophrenia may have a genetic origin (12,13,53,97), converging evidence suggests that a substantial portion of epigenetic alterations may be acquired through environmental factors (98–101). Hence, epigenetic modifications in schizophrenia may represent molecular scars of exposures to certain environmental adversities, especially when encountered during sensitive developmental periods (102–106). Consistent with this notion, a recent study showed that blood HDAC1 levels were increased in patients with schizophrenia who had been exposed to early-life stress as compared with patients with schizophrenia who had not

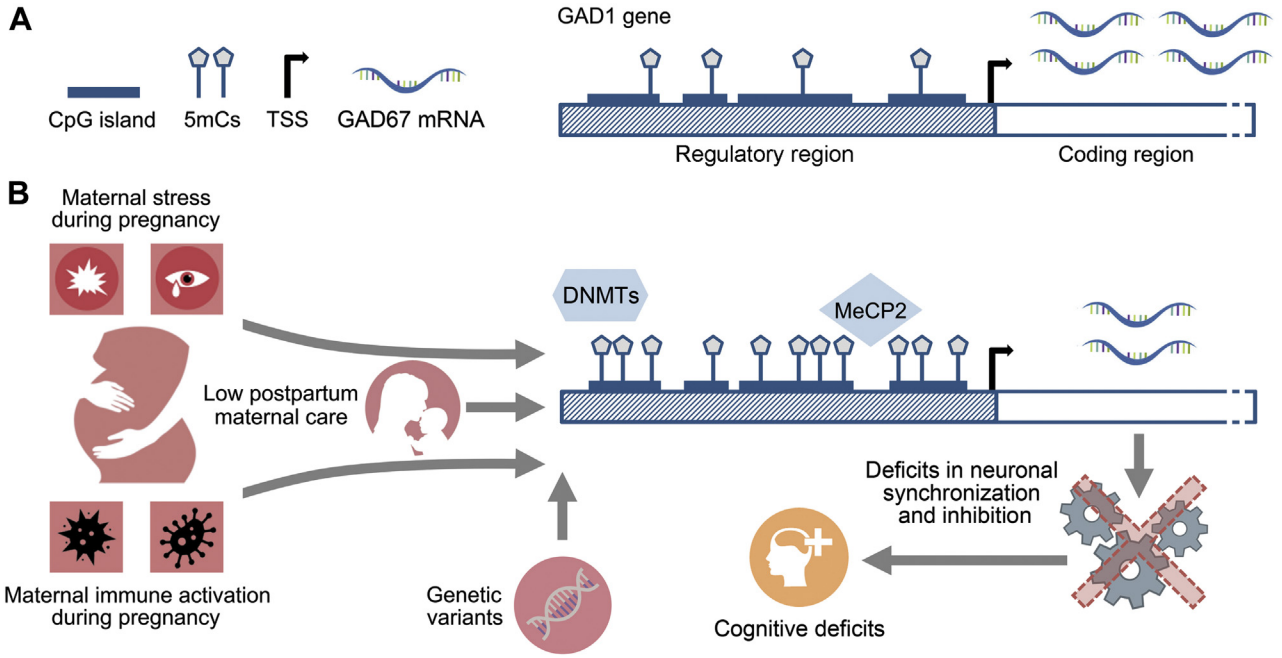

Figure 2. Convergence between environmental and genetic factors in methylation-related remodeling of the *GAD1* gene regulatory region. **(A)** Schematic illustration of a simplified version of the *GAD1* gene regulatory and coding regions that encodes the 67-kDa isoform of GAD (GAD67) mRNA. The *GAD1* gene regulatory region contains numerous CpG islands that facilitate methylation at position 5′ in the cytosine ring (5mC). **(B)** Several environmental factors, including maternal stress during pregnancy, maternal immune activation during pregnancy, and low postpartum maternal care, can cause hypermethylation of the *GAD1* gene regulatory region. Hypermethylation of the *GAD1* gene regulatory region can also be caused by certain genetic variants (e.g., rs3749034, a schizophrenia-risk single nucleotide polymorphism). In schizophrenia and disease-relevant model systems, epigenetic remodeling of the *GAD1* gene regulatory region further includes increased binding of DNMTs and MeCP2 at *GAD1* promoter regions. Together, these epigenetic changes can lead to reduced transcription of GAD67 mRNA, which in turn has been associated with impaired neuronal synchronization and inhibition and the subsequent emergence of behavioral and cognitive deficits. DNMT, DNA methyltransferase; GAD67, glutamic acid decarboxylase; MeCP2, methyl-CpG binding protein 2; mRNA, messenger RNA; TSS, transcription start site.

been exposed to early-life stress exposure (62). A plethora of findings derived from animal models, in which causal links among environmental factors, epigenetic modifications, and pathological traits can be studied against the background of genetic homogeneity, provide additional evidence for the lasting impact of environmental factors on the epigenetic machinery (107). As summarized in Table S1, many of these models are based on environmental factors implicated in the etiology of schizophrenia and related disorders (108), including exposure to prenatal or postnatal stress, inhibitors of fetal neurogenesis, infectious and noninfectious maternal immune activation (MIA) during pregnancy, gestational and postpartum nutritional deficiencies or exposure to drugs of abuse and toxicants, reduced postpartum maternal care, and chronic cannabis intake during adolescence. Not only do these models capture a broad spectrum of behavioral and neurobiological alterations pertinent to schizophrenia and other psychotic disorders, but they also show lasting abnormalities in various epigenetic profiles (Table S1).

While these models are relevant for other brain disorders as well (109), they contribute to our understanding of how exposure to environmental factors in early life can change neurodevelopmental trajectories and cause long-term brain dysfunctions implicated in schizophrenia (108,110) and beyond (109). Within the context of schizophrenia, a key feature of these models is that they allow epigenetic screens against multiple and coexisting schizophrenia-related dysfunctions while incorporating the disease-relevant concept of abnormal brain development under stringent experimental conditions. Furthermore, they can be used to explore whether pharmacological or nonpharmacological interventions targeting the epigenetic machinery are capable of attenuating abnormalities in brain development and functions (107,111–113).

Although exposure to distinct environmental factors can induce a specific set of epigenetic alterations (Table S1), there is also notable convergence between the epigenetic effects of individual environmental (and genetic) factors. Epigenetic remodeling of the gene regulatory region of *GAD1*, which encodes the 67-kDa isoform of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD67), is an illustrative example of such convergence (Figure 2). In animal models, altered DNAm in the gene regulatory region of *Gad1* and corresponding GAD67 expression deficits have been found following exposure to various environmental adversities, including exposure to MIA (114), maternal stress (112,115), and low postpartum maternal care (116). Intriguingly, these epigenetic and transcriptional changes correlate with the deficits in sociability and working memory occurring after MIA (114), emphasizing a possible functional impact of *Gad1* promoter remodeling on schizophrenia-relevant behavioral and cognitive

functions. Taken together, experimental research in animal models of early-life adversities suggests that various environmental factors, which have each been implicated in the etiology of schizophrenia and related disorders, can converge on the same molecular pathways affecting GABAergic development and functions (Figure 2).

Even though schizophrenia has been repeatedly associated with reduced GAD67 expression in cortical and hippocampal areas, it remains elusive whether this GABAergic deficit stems from altered DNAm in the gene regulatory region of *GAD1* (20,35,39,46,102,117). Several schizophrenia postmortem studies, however, show altered expression and/or promoter binding of enzymes that are critical for the maintenance of the DNA methylation/demethylation equilibrium of cytosines positioned within a CG context, including DNMTs, TET proteins, and MeCP2 (118–121). In addition, other epigenetic changes may contribute to *GAD1* promoter remodeling in schizophrenia as well, including reduced repressive chromatin-associated DNAm at the promoter, decreased H3K4 trimethylation (a marker of active transcription), and changes in the 3D configuration of the linear sequence containing the *GAD1* locus (20,119,122,123).

While exposure to environmental adversities may readily contribute to epigenetically driven deficits in GABAergic gene transcription (Figure 2), several gene variants appear to do so as well (53,97,124,125). The latter effects are consistent with the considerable genetic influence on schizophrenia risk (2,5). Future studies are warranted, however, to explore how specific environmental and genetic factors can have additive or interactive effects on molecular pathways affecting GABAergic gene transcription in schizophrenia and related disorders.

EPIGENETIC MODIFICATIONS AS SOURCE OF PHENOTYPIC VARIABILITY

Schizophrenia is known to be highly heterogeneous in terms of both its clinical presentation and its etiology. Even though they are practical for clinicians, the diagnostic systems currently used are not well equipped to capture the clinical heterogeneity of the disorder(s) that Bleuler initially referred to as the "group of schizophrenias" [see (126)]. The clinical heterogeneity of schizophrenia is also mirrored by the underlying genetic risk, which appears to be polygenic and highly heterogeneous. While both common and rare genetic variants shape the risk of developing schizophrenia (3,4), the disorder's common genetic risk is usually indexed by the PRS, which reflects the cumulative sum of risk-associated alleles at common variants across the entire genome (4).

Under certain conditions, the PRS of schizophrenia may be useful for estimating individual disease risk (127), treatment response (128), and certain symptoms (129), but it might not be capable of fully seizing the biological basis of the disorder's clinical and etiological heterogeneity (71). For example, in a recent brain imaging study, it was found that the PRS was unrelated to the substantial brain structural heterogeneity present in schizophrenia (130). These findings suggest that brain variability in schizophrenia results from interactions between environmental and genetic factors that are not captured by the PRS.

To what extent may epigenetic modifications contribute to phenotypic heterogeneity in schizophrenia? We deem this a very likely possibility for various reasons. First, environmental factors can influence the phenotypic presentation of schizophrenia even if they do not interact with common genetic risk of the disorder. One example is prenatal exposure to infectious or noninfectious MIA, which has been shown to influence structural brain abnormalities and executive functioning in schizophrenia (131,132). While direct involvement of epigenetic modifications still awaits examination in these epidemiological associations, findings from animal models show that such modifications provide a key mechanism mediating the effects of MIA on schizophrenia-related structural and functional brain anomalies in the offspring (114,133,134) (see also Table S1). Interestingly, the specificity of epigenetic modifications and behavioral abnormalities induced by MIA are dependent on the precise prenatal stage at which MIA occurs (133), highlighting that epigenetic variability mirrors phenotypic variability, at least in the context of MIA. Second, genetic variants that are not considered as common in the genetics of schizophrenia may nevertheless be etiologically relevant and contribute to phenotypic heterogeneity by interacting with environmentally acquired epigenetic modifications. The interaction between DISC1 and adolescent stress exposure, which is mediated by stress-induced epigenetic modifications in the mesocortical dopamine system (135), is an illustrative example of this notion. Third, in healthy subjects, the broad interindividual variability in PFC development and functions is mirrored by variable epigenetic profiles, which partly interact with genetic factors (136,137). Fourth, even if some epigenetic modifications can be stable and perpetuate across generations, others are highly dynamic and reversible (138). The latter not only provides a rationale for epigenetically acting treatments (111–113,139) but also provides a parsimonious explanation for the variable and often irreproducible epigenetic findings in schizophrenia (Tables 1 and 2). Taken together, epigenetic modifications not only may help to explain missing heritability in schizophrenia but also are a likely source of the disorder's phenotypic heterogeneity (Figure 3).

LIMITATIONS AND FUTURE DIRECTIONS

Even though the evidence for a role of abnormal epigenetic processes in schizophrenia has rapidly accumulated over recent years (140), there is as yet no clear picture of the precise disease mechanisms involved. Indeed, different genome-wide studies have limited overlap in terms of their results, and these unbiased approaches could replicate only a small fraction of the initial findings that were obtained from targeted (hypothesis-driven) epigenetic studies. The lack of reproducibility is most likely accounted for by a number of factors, including small sample size and study population bias (141), influence of chronic pharmacotherapy (142), and other confounders such as smoking (143), methodological differences in epigenetic screening (144), and heterogeneity of cell populations studied (145). The latter has emerged as an important source of data variability at both the gene transcriptional and epigenetic levels (145), which in turn can mask diagnostic differences or lead to spurious and irreproducible findings. Accounting for cell

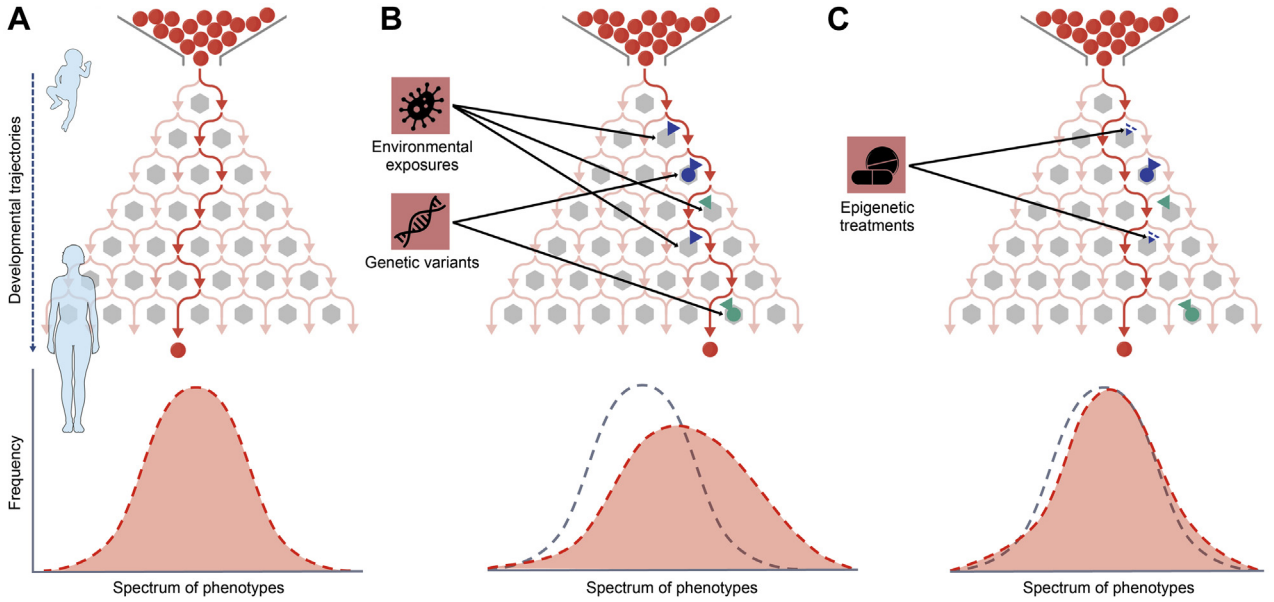

Figure 3. Hypothetical model of the epigenetic influence on phenotypic trait variability. **(A)** Using the bean machine (Galton Board) as a simplified metaphor for the specification of developmental trajectories, each developmental step is influenced by stochastic events occurring at distinct stages of development. With the pegs representing developmental specification nodes, developmental steps can bounce either left or right when hitting the pegs. The phenotypic traits associated with the developmental trajectories are spread according to a certain probability distribution. The current example shows a normal distribution of phenotypic traits, which would occur when running a large number of identical balls through a leveled device with unbiased pegs. **(B)** In this hypothetical model, environmentally and/or genetically driven epigenetic modifications targeting the pegs (i.e., the developmental specification nodes) can bias them toward one direction, which in turn changes the probability distribution of the eventual phenotypic traits. Note that the influence of epigenetic modifications on biasing the developmental trajectories would depend on its developmental timing and its specificity as well as on the nature of the developmental specification node it targets (not shown). **(C)** Pharmacological or nonpharmacological interventions targeting epigenetic modifications at accessible developmental specification nodes may reshape the probability distribution of phenotypic traits.

heterogeneity in epigenetic studies will require the implementation of single-cell technologies (146) and/or the use of statistical methods that compute the relative proportions of cell types from heterogeneous bulk tissues (147). The establishment of large databases and publicly available resources of multidimensional genomic and epigenetic data obtained from tissue-specific and cell type–specific samples, such as those provided by the PsychENCODE initiative (68), will critically help unravel the complex nature of genetic and epigenetic abnormalities in schizophrenia and other chronic brain disorders. Such initiatives also underline the importance of incorporating multiple and coexisting epigenetic anomalies in molecular disease models of schizophrenia and related disorders rather than relying on single genes such as *GAD1* (Figure 3). Indeed, similar to the polygenic architecture of schizophrenia (2,5,127–129), a multitude of genes are likely to be epigenetically dysregulated in this disorder and in response to environmental risk factors such as prenatal infection (133).

Most of the available epigenetic findings in schizophrenia are based on analyses of postmortem brain samples, which imposes the additional challenge of retrieving a reliable patient history or antemortem diagnosis (148). The use of postmortem samples also precludes the identification of dynamic changes in epigenetic signatures that may occur in response to certain clinical manifestations (e.g., acute exacerbation of psychotic symptoms) or pharmacological treatments. Another limitation of postmortem analyses is that they are unlikely to provide a satisfactory answer to the question of whether epigenetic anomalies are the cause or consequence of the disease. Addressing this question in the context of schizophrenia warrants the use of longitudinal studies in which epigenetic signatures can be followed-up in the same subjects from premorbid and high-risk states to the eventual onset of full-blown psychosis. Such longitudinal investigations, however, will require the use of easily accessible tissues such as peripheral blood, saliva, and olfactory epithelium (149). Even if epigenetic signatures in peripheral tissues might not necessarily capture disease-associated epigenetic changes occurring in the CNS (Tables 1 and 2), they can provide valuable information regarding the clinical course of schizophrenia, including conversion from a high-risk state to first-onset psychosis (50). The complementary use of animal models will further aid the interpretation of epigenetic findings obtained from longitudinal studies in humans. Indeed, not only do animal models allow for a comparison of epigenetic signatures in peripheral and CNS tissues at various stages of development (133), but they also enable experimental testing of target sequences affected by chromatin alterations and their effects on brain development and functions (150).

CONCLUSIONS

Despite the current limitations in the field, it is increasingly recognized that epigenetic modifications may play a critical

role in the etiology and pathophysiology of schizophrenia. Recent findings suggest that certain schizophrenia risk loci can influence stochastic variation in gene expression through epigenetic processes, highlighting the intricate interaction between the genetic control and epigenetic control of developmental trajectories in schizophrenia. In addition, a substantial portion of epigenetic alterations in schizophrenia may be acquired through environmental factors and remain stable as molecular scars. Some of these scars may influence brain functions throughout the entire lifespan and may even be transmitted across generations via epigenetic germline inheritance (see Supplement). Moreover, epigenetic modifications are a plausible molecular source of phenotypic heterogeneity and offer a target for therapeutic interventions. The further elucidation of epigenetic modifications may thus increase our knowledge regarding schizophrenia's heterogeneous etiology and pathophysiology and, in the long term, may advance personalized treatments through the use of biomarker-guided epigenetic interventions.

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ARTICLE INFORMATION

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